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Short Communications

Increased dystrophin mRNA and protein levels in atrophic skeletal muscles in streptozotocin-induced diabetic rat

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Running Title: Dystrophin and muscle atrophy in diabetes

Abstract

Severe diabetes frequently induces skeletal muscle atrophy, and dystrophin disruption has been implicated in the pathogenesis of skeletal muscle atrophy. We hypothesized that the downregulation of dystrophin expression causes diabetic-induced muscle atrophy, and investigated whether dystrophin mRNA and protein levels are altered in the atrophic muscles of diabetic rats. Rats received a single intravenous injection of streptozotocin (STZ) (45 mg/kg body weight). Slow-twitch soleus and fast-twitch extensor digitorum longus muscles were dissected from each rat 4 or 12 weeks after the STZ injection. The STZ group had significantly higher blood glucose levels and lower body weights than the control group. The relative muscle weight per body weight was also lower in the STZ group than in the control group, and these changes accompanied a reduction in glucose transporter 4. The phosphorylation of Akt Ser^{473} and p70 S6 kinase Thr^{389} was lower in the soleus and extensor digitorum longus muscles of the diabetic rats than in those of the control rats. In contrast, dystrophin mRNA and protein expression were higher in the muscles of the diabetic rats than in those of the control rats. A histochemical study showed that the localization of dystrophin did not differ between the muscles of the control and diabetic rats. Our data suggest that the downregulation of dystrophin is not a general characteristic associated with skeletal muscle in diabetes.

Keywords: dystrophin, atrophic muscle, hyperglycemia, insulin, streptozotocin

Abstract

重度の糖尿病状態では進行性の骨格筋萎縮を発症することが報告されている。また骨格筋構成蛋白質の 1 つであるジストロフィンの欠損は骨格筋萎縮を誘発する。本研究では糖尿病性筋萎縮の進行にはジストロフィン欠損が関与しているという仮説を立て、筋萎縮を呈するストレプトゾトシン（STZ）誘発性糖尿病モデル動物を用いて、萎縮骨格筋におけるジストロフィン mRNA およびタンパク発現量を測定した。8 週齢の雄性 Sprague-Dawly ラットに生理食塩水に溶解した STZ（45 mg/kg body weight）を尾静脈投与した群を STZ 群とし、生理食塩水を投与した群を対照群とした。投与 4 または 12 週間後にヒラメ筋および長指伸筋を摘出するとともに血液を採取した。STZ 群では対照群に対し、血糖値の上昇および体重減少、体重あたりのヒラメ筋および長指伸筋重量の低下が認められた。また筋萎縮に伴って glucose transporter 4 タンパク発現量の減少が認められた。STZ 群の萎縮骨格筋では対照群に比べ、Akt Ser^{473} リン酸化、p70 S6 kinase Thr^{389} リン酸化が減少した。一方、ジストロフィン mRNA およびタンパク発現量がともに増加した。免疫組織化学的検討の結果から、ジストロフィンの局在は両群に差はなかった。以上の結果から、糖尿病状態における骨格筋萎縮にジストロフィン欠損は関与していないことが示唆される。

江川達郎、増田慎也、後藤勝正、林達也

Introduction

Dystrophin is a structural protein that connects the cytoskeleton of a muscle fiber to the surrounding extracellular matrix through the cell membrane. Mutation of dystrophin disrupts the mechanical linkage and signaling pathway, resulting in membrane damage, necrosis, and eventual muscle atrophy [1)]. Dystrophin dysfunction is also implicated in muscle wasting of cancer cachexia [2, 3)], which is associated with impaired insulin action in skeletal muscle [4)].

Poorly controlled diabetes affects protein metabolism [5)] and induces skeletal muscle atrophy [6)]. Although several studies have shown that reduced protein synthesis contributes to it [7, 8)], the precise mechanism of diabetic-induced muscle atrophy is not understood. Using streptozotocin (STZ)-induced diabetic rats, recent studies have demonstrated that reduced Akt/ mammalian target of rapamycin (mTOR) signaling is associated with diabetic-induced muscle atrophy [9)]. Risson et al. [10)] showed that the inactivation of mTOR leads to advanced myopathy, with a strong reduction in the muscle dystrophin content.

We hypothesized that the downregulation of dystrophin expression might be associated with diabetic-induced muscle atrophy. Thus, the primary purpose of this study was to examine the expression of dystrophin mRNA and protein in the atrophic muscles of STZ-induced diabetic rats. We also examined the changes in dystrophin localization in these rats by histochemical analysis.

Materials and methods

Animals

All protocols for animal use and euthanasia followed the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences (Physiological Society of Japan), in accordance with international guidelines, and were approved by the Kyoto University Graduate School of Human and Environmental Studies. Male Sprague Dawley rats (8 weeks old) were housed in an animal room maintained at 22–24 °C on a 12:12-h light–dark cycle and were fed a standard laboratory diet and water ad libitum.

STZ treatment

Diabetes was induced with STZ (Sigma, St Louis, MO, USA). In brief, the animals received a single intravenous injection of STZ (45 mg/kg body weight) via the tail vein. The control animals received an equivalent volume of saline. Blood glucose levels were measured using the glucose oxidase method, with an automated blood glucose analyzer (GluTestAce, Sanwa Chemical Research Institute Co., Inc., Nagoya, Japan). Diabetes was defined as a nonfasting glucose level of ≥ 300 mg/dl.

Muscle sampling

The animals were anesthetized with pentobarbital sodium (50 mg/kg body weight) and killed 4 or 12 weeks after the STZ or saline injection. The soleus (SOL) and extensor digitorum longus (EDL) muscles were dissected from each rat, weighed, immediately frozen in liquid nitrogen, and stored at –80 °C for immunoblotting and Real-time RT-PCR analysis. Some muscles were frozen in 2-methylbutane cooled with solid CO_2 and stored at –80 °C for histochemical analysis.

103 Immunoblotting analysis

104 Total protein from muscles were extracted as we described previously [11, 12] and subjected
105 to immunoblotting analyses. Blots were incubated with rabbit-anti Akt Ser^{473} (9271, Cell
106 Signaling Technology, Danvers, MA), rabbit-anti Akt (9272, Cell Signaling), rabbit-anti p70
107 S6 kinase (p70S6K) Thr^{389} (9205, Cell Signaling Technology, Danvers, MA), rabbit-anti
108 p70S6K (9202, Cell Signaling), rabbit anti-glucose transporter (GLUT) 4 (AB1346;
109 Chemicon, Temecula, CA, USA), mouse anti-dystrophin antibody (MAB1692; Chemicon).
110 The signal was detected by using ECL reagents (GE Healthcare, Buckinghamshire, UK). The
111 intensity of the signals was quantified using MultiAnalyst software (Bio-Rad Laboratories,
112 Berkeley, CA, USA). Every blot was duplicated and the mean value was adopted for each
113 sample.

114

115 Real-time RT-PCR analysis

116 Real-time RT-PCR analysis was performed according to Yasuhara et al. [13]. Briefly, total
117 RNA was extracted from muscles using the miRNeasy Mini kit (Qiagen, Hiden, Germany).
118 Samples were reverse-transcribed using PrimeScript RT Master Mix (Perfect Real Time) for
119 mRNA (Takara Bio, Otsu, Japan). Synthesized cDNA was applied to real-time reverse
120 transcription-PCR (Thermal Cycler Dice Real Time System IIMRQ, Takara Bio) using Takara
121 SYBR Premix Ex Taq II for mRNA, and analyzed with Takara Thermal Cycler Dice Real
122 Time System Software Ver. 4.00. To normalize the amount of total RNA present in each
123 reaction, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA for mRNA was used
124 as an internal standard. The primers were designed by using the Takara Bio Perfect Real Time
125 Support System (Takara Bio). Primers used for detection of rat cDNA were as follows:
126 Dystrophin, 5'-AGGCCAAGTGTAACATCTGTAAGGA-3' (forward) and
127 5'-CCTTAGCAACTCGGCCAGAA-3' (reverse); GAPDH,

5’-GGCACAGTCAAGGCTGAGAATG-3’ (forward) and 5’-ATGGTGGTGAAGACGCCAGTA-3’ (reverse).

Histochemical analysis

The histochemical analyses used to detect dystrophin were as we described previously [11]. The sections were incubated with mouse anti-dystrophin antibody (MAB1692; Chemicon), and stained with 3,3’-diaminobenzidine. Histochemical analysis was performed using a microscope (Optiphot-2, Nikon, Tokyo, Japan) with a charge-coupled device camera and a computerized image processing system (Image/J 1.36b). To calculate the mean cross-sectional area of the muscle fibers, 100-150 fibers were randomly picked up from each of the muscles.

Statistical analysis

The results are presented as means ± SE. Means were compared with two-way ANOVA, followed by a post hoc comparison with Scheffé’s test as appropriate. $P < 0.05$ was considered statistically significant.

Results

Glucose levels, body weight, muscle weight, GLUT4 levels

The STZ group had significantly higher blood glucose levels and lower body weights than the control group at 4 and 12 weeks (Table 1). The muscle weight of the STZ group was significantly lower than that of the control group at 4 and 12 weeks. The relative muscle weight per body weight was also lower in the STZ group than in the control group, except for the SOL at 12 week (P=0.08).

It has been shown that GLUT4 expression is greatly reduced in some conditions leading to muscle atrophy such as disuse [14], aging [15], denervation [16], and diabetes [17]. In accordance with these studies, the GLUT4 levels in SOL and EDL muscles were reduced in the STZ group compared with the control group (Figure 1).

Akt and p70S6K phosphorylation

Akt Ser^{473} phosphorylation was significantly reduced in the SOL and EDL muscles of the STZ group at 12 weeks (Figure 2A). The change at 4 weeks was not statistical significant (Figure 2A). p70S6K is major substrate for mTOR, and the phosphorylation of p70S6K Thr^{389} in the STZ group was clearly reduced compared with that in the control group at both 4 and 12 weeks (Figure 2B).

Dystrophin mRNA and protein levels

The dystrophin mRNA and protein levels were higher in the SOL of the STZ group than in the control group at both 4 and 12 weeks, and the mRNA and protein levels were also higher in the EDL of the STZ group than in the control group at 12 weeks (Figure 3A and 3B).

A histochemical analysis showed marked fiber atrophy in the 12 weeks diabetic group compared with the control group (SOL, control = 4807 ± 275 μm^2 vs. STZ = 2060 ± 106;

EDL, control = 3305 ± 187 vs. STZ = 1144 ± 38, $p<0.01$, respectively; n = 4 per group), but dystrophin was expressed at the sarcolemma of all muscle fibers in the SOL and EDL muscles of the STZ and control groups at 12 weeks (Figure 3C).

Discussion

The STZ-induced diabetic animal model has often been used to study muscle atrophy in diabetes [9, 18, 19]. We have shown for the first time that the dystrophin mRNA and protein levels are increased in the atrophic muscles of STZ-induced diabetic rats (Figure 3A), which are characterized by insulin-deficient hyperglycemia, low body weight, and low muscle weight (Table 1). Low muscle weight per body weight (Table 1) and a reduction in GLUT4 levels (Figure 1) indicate that skeletal muscle atrophy was induced in our diabetic rats, because low GLUT4 expression is seen in some atrophic muscles including STZ-induced diabetes [14-17]. Contrary to our findings, Mulvey et al. [20] reported that the expression of dystrophin is reduced in the skeletal muscles of type 2 diabetic Goto-Kakizaki rats. They speculated that the loss of dystrophin is at least partly responsible for the abnormal characteristics of the skeletal muscle in diabetes. However, their diabetic rats did not exhibit structural abnormalities, such as muscle atrophy.

Although the exact function of dystrophin is not completely understood, it is known to play an important role in regulating the structural stability of the muscle cell membrane [1, 21]. The signal transduction cascade from dystrophin has also been implicated in cell survival and defense mechanisms, and in the regulation of the balance between cell survival and cell death [21, 22]. There is also a functional link between dystrophin and cell growth signaling [10]. It is noteworthy that dystrophin is elevated at the sarcolemma of cardiac myocytes in failing myocardium, and this is interpreted as a mechanism to preserve cellular stability and integrity [23]. Therefore, the increased expression of dystrophin might be a compensatory response that defend the muscle structure and functions against muscle atrophy under severely diabetic conditions. Further study is required to clarify the role of dystrophin upregulation in STZ-induced diabetes.

Consistent with a previous study that demonstrated that Akt/mTOR signaling is

associated with diabetic-induced muscle atrophy [9], we confirmed that Akt Ser^{473} and p70S6K Thr^{389} phosphorylation are reduced in atrophic muscles during diabetes (Figure 2). It has been demonstrated that the inactivation of mTOR leads to advanced myopathy, with a strong reduction in the muscle dystrophin content [10]. However, we found that dystrophin levels were upregulated in the atrophic muscles of diabetic rats (Figure 3) although Akt/mTOR/p70S6K signaling was downregulated (Figure 2). This implies that the downregulation of mTOR signaling does not always cause a reduction in the dystrophin content of muscles.

Taken together, our data suggest that a reduced dystrophin content is not a common feature of atrophic skeletal muscle, and that the pathogenesis of diabetic-induced muscle atrophy cannot be attributed to the downregulation of dystrophin levels. Other mechanisms such as the disturbance of protein synthesis signaling [9] and the increased expression of genes involved in protein degradation [24] may be crucial modulators of diabetic muscle atrophy.

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References

1. Lapidos KA, Kakkar R, and McNally EM. 2004. The dystrophin glycoprotein complex: signaling strength and integrity for the sarcolemma. *Circ Res* 94: 1023-31.

2. Acharyya S, Butchbach ME, Sahenk Z, Wang H, Saji M, Carathers M, Ringel MD, Skipworth RJ, Fearon KC, Hollingsworth MA, Muscarella P, Burghes AH, Rafael-Fortney JA, and Guttridge DC. 2005. Dystrophin glycoprotein complex dysfunction: a regulatory link between muscular dystrophy and cancer cachexia. *Cancer Cell* 8: 421-32.

3. Acharyya S and Guttridge DC. 2007. Cancer cachexia signaling pathways continue to emerge yet much still points to the proteasome. *Clin Cancer Res* 13: 1356-61.

4. Honors MA and Kinzig KP. 2012. The role of insulin resistance in the development of muscle wasting during cancer cachexia. *J Cachexia Sarcopenia Muscle* 3: 5-11.

5. Gougeon R, Pencharz PB, and Sigal RJ. 1997. Effect of glycemic control on the kinetics of whole-body protein metabolism in obese subjects with non-insulin-dependent diabetes mellitus during iso- and hypoenergetic feeding. *Am J Clin Nutr* 65: 861-70.

6. Chokroverty S, Reyes MG, Rubino FA, and Tonaki H. 1977. The syndrome of diabetic amyotrophy. *Ann Neurol* 2: 181-94.

7. Flaim KE, Copenhaver ME, and Jefferson LS. 1980. Effects of diabetes on protein synthesis in fast- and slow-twitch rat skeletal muscle. *Am J Physiol* 239: E88-95.

8. Pain VM, Albertse EC, and Garlick PJ. 1983. Protein metabolism in skeletal muscle, diaphragm, and heart of diabetic rats. *Am J Physiol* 245: E604-10.

9. Hulmi JJ, Silvennoinen M, Lehti M, Kivela R, and Kainulainen H. 2012. Altered REDD1, myostatin, and Akt/mTOR/FoxO/MAPK signaling in streptozotocin-induced diabetic muscle atrophy. *Am J Physiol Endocrinol Metab* 302: E307-15.

10. Risson V, Mazelin L, Roceri M, Sanchez H, Moncollin V, Corneloup C, Richard-Bulteau H, Vignaud A, Baas D, Defour A, Freyssenet D, Tanti JF,

239 Le-Marchand-Brustel Y, Ferrier B, Conjard-Duplany A, Romanino K, Bauche S, Hantai D,
240 Mueller M, Kozma SC, Thomas G, Ruegg MA, Ferry A, Pende M, Bigard X, Koulmann N,
241 Schaeffer L, and Gangloff YG. 2009. Muscle inactivation of mTOR causes metabolic and
242 dystrophin defects leading to severe myopathy. *J Cell Biol* 187: 859-74.
243 11. Masuda S, Hayashi T, Hashimoto T, and Taguchi S. 2009. Correlation of
244 dystrophin-glycoprotein complex and focal adhesion complex with myosin heavy chain
245 isoforms in rat skeletal muscle. *Acta Physiol (Oxf)* 195: 483-94.
246 12. Egawa T, Hamada T, Kameda N, Karaike K, Ma X, Masuda S, Iwanaka N, and
247 Hayashi T. 2009. Caffeine acutely activates 5'adenosine monophosphate-activated protein
248 kinase and increases insulin-independent glucose transport in rat skeletal muscles.
249 *Metabolism* 58: 1609-17.
250 13. Yasuhara K, Ohno Y, Kojima A, Uehara K, Beppu M, Sugiura T, Fujimoto M, Nakai
251 A, Ohira Y, Yoshioka T, and Goto K. 2011. Absence of heat shock transcription factor 1
252 retards the regrowth of atrophied soleus muscle in mice. *J Appl Physiol* 111: 1142-1149.
253 14. Blakemore SJ, Rickhuss PK, Watt PW, Rennie MJ, and Hundal HS. 1996. Effects of
254 limb immobilization on cytochrome c oxidase activity and GLUT4 and GLUT5 protein
255 expression in human skeletal muscle. *Clin Sci (Lond)* 91: 591-9.
256 15. Stump CS, Tipton CM, and Henriksen EJ. 1997. Muscle adaptations to hindlimb
257 suspension in mature and old Fischer 344 rats. *J Appl Physiol* 82: 1875-81.
258 16. Coderre L, Monfar MM, Chen KS, Heydrick SJ, Kurowski TG, Ruderman NB, and
259 Pilch PF. 1992. Alteration in the expression of GLUT-1 and GLUT-4 protein and messenger
260 RNA levels in denervated rat muscles. *Endocrinology* 131: 1821-5.
261 17. Han XX, Fernando PK, and Bonen A. 2000. Denervation provokes greater reductions
262 in insulin-stimulated glucose transport in muscle than severe diabetes. *Mol Cell Biochem* 210:
263 81-9.

18. Kelleher AR, Fairchild TJ, and Keslacy S. 2010. STZ-induced skeletal muscle atrophy is associated with increased p65 content and downregulation of insulin pathway without NF-kappaB canonical cascade activation. *Acta Diabetol* 47: 315-23.

19. Roberts-Wilson TK, Reddy RN, Bailey JL, Zheng B, Ordas R, Gooch JL, and Price SR. 2010. Calcineurin signaling and PGC-1alpha expression are suppressed during muscle atrophy due to diabetes. *Biochim Biophys Acta* 1803: 960-7.

20. Mulvey C, Harno E, Keenan A, and Ohlendieck K. 2005. Expression of the skeletal muscle dystrophin-dystroglycan complex and syntrophin-nitric oxide synthase complex is severely affected in the type 2 diabetic Goto-Kakizaki rat. *Eur J Cell Biol* 84: 867-83.

21. Gumerson JD and Michele DE. 2011. The dystrophin-glycoprotein complex in the prevention of muscle damage. *J Biomed Biotechnol* 2011: 1-13.

22. Rando TA. 2001. The dystrophin-glycoprotein complex, cellular signaling, and the regulation of cell survival in the muscular dystrophies. *Muscle Nerve* 24: 1575-94.

23. Schaper J, Kostin S, Hein S, Elsasser A, Arnon E, and Zimmermann R. 2002. Structural remodelling in heart failure. *Exp Clin Cardiol* 7: 64-8.

24. Lecker SH, Jagoe RT, Gilbert A, Gomes M, Baracos V, Bailey J, Price SR, Mitch WE, and Goldberg AL. 2004. Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *Faseb J* 18: 39-51.

Table 1. Body weight, plasma glucose concentration, muscle weight in control and STZ-induced diabetic rats.

	Control			STZ		
Period (week)	0	4	12	0	4	12
Body weight (g)	191 ± 4	334 ± 8	531 ± 18	190 ± 4	280 ± 4 †	276 ± 4 †
Plasma glucose (mg/dL)	56 ± 2	76 ± 5	76 ± 3	70 ± 6	476 ± 22 †	490 ± 22 †
SOL						
muscle weight (mg)	—	140 ± 5	221 ± 4	—	100 ± 4 †	107 ± 5 †
weight/body weight	—	0.42 ± 0.011	0.42 ± 0.016	—	0.36 ± 0.010 *	0.38 ± 0.012
EDL						
muscle weight (mg)	—	154 ± 4	217 ± 6	—	94 ± 10 †	87 ± 5 †
weight/body weight	—	0.46 ± 0.016	0.40 ± 0.016	—	0.33 ± 0.024 †	0.32 ± 0.012 †
number of animals	14	4	10	12	3	9

Values are mean ± SE.

* P<0.05, † P<0.01 Significantly different from corresponding control group.

Figure legend

Figure 1

GLUT4 levels in SOL and EDL muscle. The muscle isolated from control and STZ groups was subjected to immunoblot analysis. Fold increases are expressed relative to the level of signal in the muscle of normal group (4 weeks). Equal protein loading and transfer was confirmed by Coomassie brilliant blue (CBB) staining of a 200-kDa protein. Representative immunoblots are shown. Values are mean ± SE. *P<0.05, †P<0.01 vs. corresponding control group.

Figure 2

Akt Ser^{473} phosphorylation (A), p70S6K Thr^{389} phosphorylation (B) levels in SOL and EDL muscle. The muscle isolated from control and STZ groups was subjected to immunoblot analysis. Fold increases are expressed relative to the level of signal in the muscle of normal group (4 weeks). Representative immunoblots are shown. Values are mean ± SE. *P<0.05, †P<0.01 vs. corresponding control group.

Figure 3

Dystrophin mRNA (A) and protein (B) levels in SOL and EDL muscle. The muscle isolated from control and STZ groups was subjected to real-time RT-PCR and immunoblot analysis. Fold increases are expressed relative to the levels in the muscle of normal group (4 weeks). Equal protein loading and transfer for immunoblot analysis was confirmed by CBB staining of a 200-kDa protein. Representative immunoblots are shown. Values are mean ± SE. *P<0.05, †P<0.01 vs. corresponding control group. Histochemical analysis of dystrophin localization (C). Representative transverse sections of SOL and EDL muscle dissected from 12-week experimental groups are shown. Scale bars indicate 25 μm.

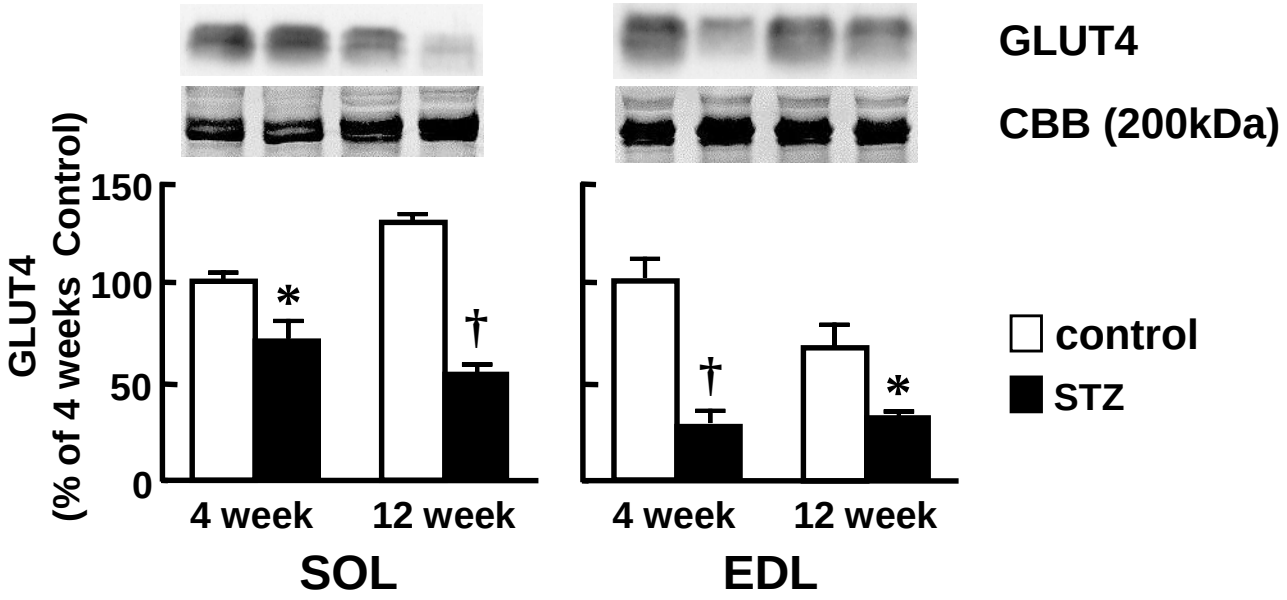

Figure 1

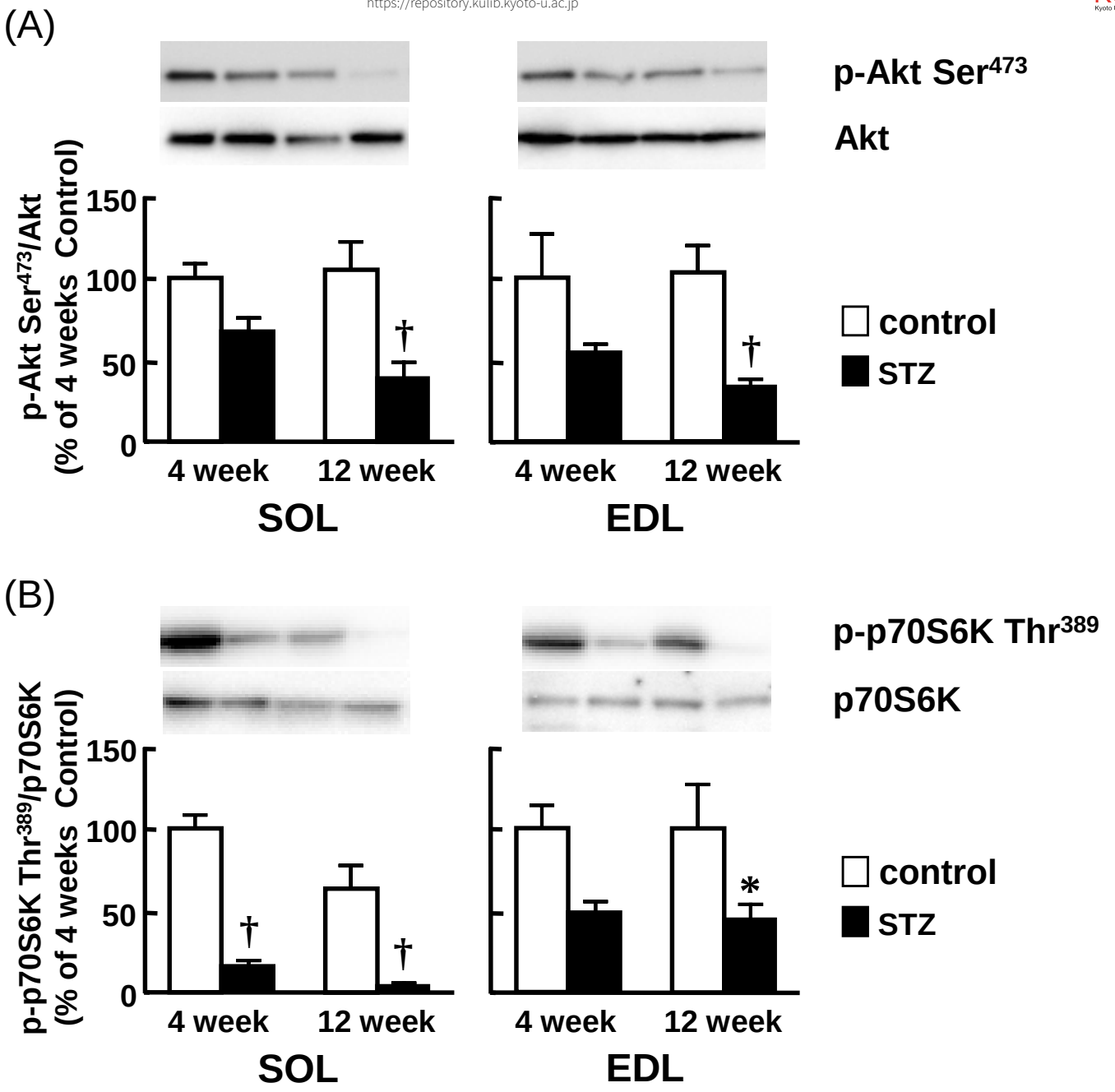

Figure 2

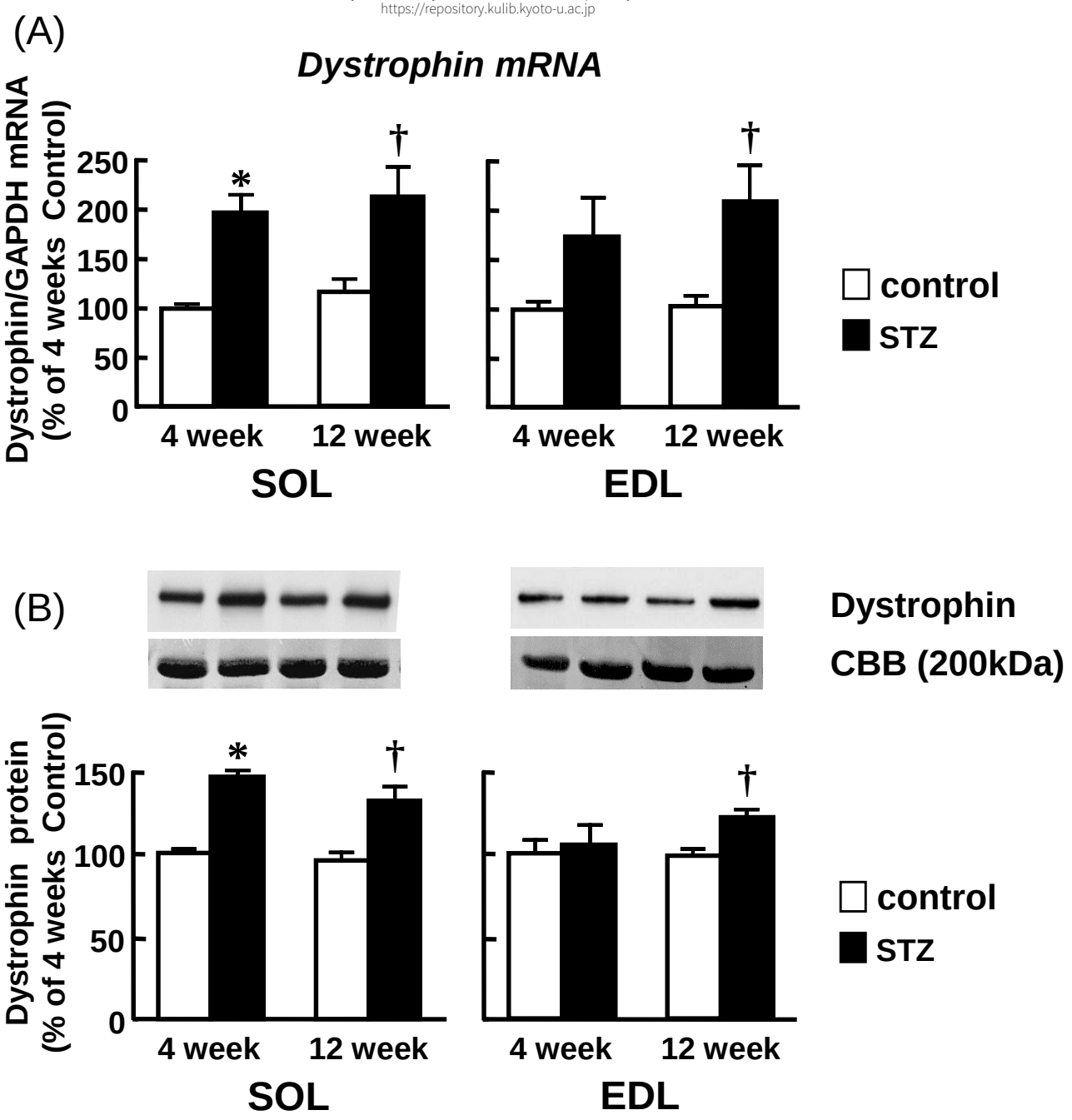

Figure 3

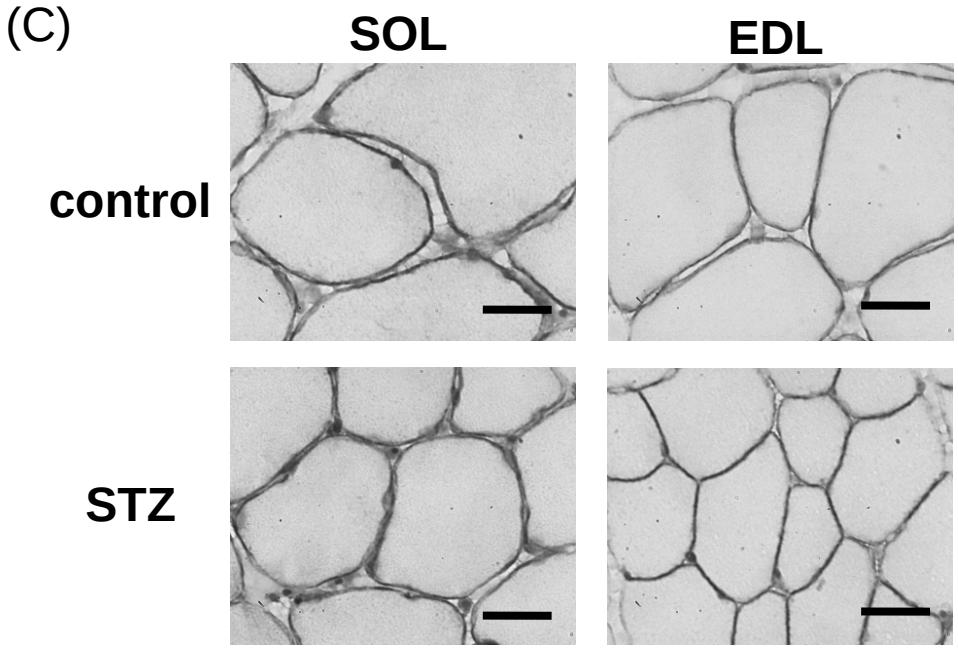

Figure 3